1.0 Purpose and Scope

1.1 This procedure describes the preparation of samples for headspace gas chromatography analysis for ethyl alcohol.

1.2 Samples to be prepared may include calibration standards, whole blood, and blood samples which are thought to contain alcohol.

1.3 The scope of this SOP includes preparation of the internal standard, timing mix and calibration standards, retrieving and opening of evidentiary samples, preparation of vials for analysis, paperwork and documentation.

2.0 Responsibility

2.1 All analysts having the responsibility of preparing samples for alcohol content analysis are responsible for following this procedure as written.

2.2 This procedure is reviewed periodically by the Alcohol Program staff. Revisions are made at that time or when there is an identified need to change this written procedure to be compatible with changing needs in the analytical process.

2.3 All analysts performing this procedure for the purpose of preparing samples for forensic analyses must be fully trained and demonstrate proficiency in the use of this procedure annually.

3.0 Procedure Steps (Procedure performed in room 266)

3.1 Reagents

3.1.1 Ethanol (Pharmco 200 proof ACS/USP grade)

3.1.2 Acetaldehyde (ACS reagent grade)

3.1.3 Acetone (ACS reagent grade)

3.1.4 Isopropanol (ACS reagent grade)

3.1.5 Methanol (ACS reagent grade)

3.1.6 NaCl (USP grade)

3.1.7 N-Propanol (ACS reagent grade)

3.1.8 t-Butanol (ACS reagent grade)

3.1.9 $CuSO_4$ (ACS reagent grade)

3.1.10 diH_2O water

3.2 Apparatus

3.2.1 Class A volumetric flasks

3.2.2 Glass vials with septum and screw caps.

3.2.3 Pasteur pipettes

3.2.4 1L bottle

3.2.5 Analytical Balance

3.2.6 Vortexer

3.2.7 Syringe

3.2.8 250ul, 500ul, & 1000ul fixed volume and 200-1000uL pipettes

3.2.9 Pipette tips

3.2.10 Wide bore, aerosol barrier pipette tips

3.2.11 20ml round bottom headspace auto sampler vials

3.2.12 Septum caps

3.2.13 Crimping tool

3.2.14 Test tube and vial racks

3.3 Internal Standard Preparation

3.3.1 Add approximately 500ml of diH_2O to a 1L volumetric flask.

3.3.2 Add 20g NaCl.

3.3.3 Add 0.5g N-Propanol.

3.3.4 Add 0.15g t-Butanol.

3.3.5 Add ~ 0.5g $CuSO_4$.

3.3.6 Bring to volume with diH_2O and shake well to dissolve solids.

3.3.7 Transfer to a labeled 1L bottle.

3.3.8 Document the solution in the Reagent Preparation Log.

3.3.8.1 The lot number is IS-MMDDYYYY.

3.3.8.2 Solution expires one year from date of preparation.

3.4 Timing Mix Preparation

3.4.1 Add a small amount of diH_2O to a 100mL volumetric flask.

3.4.2 Add ~0.06g Acetaldehyde.

3.4.3 Add ~0.12g Methanol.

3.4.4 Add ~0.06g Acetone.

3.4.5 Add ~0.08g Isopropanol.

3.4.6 Add ~0.08g Ethanol.

3.4.7 Bring to volume with diH_2O.

3.4.8 Transfer to a glass vial with a septum and screw cap. Label the vial.

3.4.9 Document the solution in the Reagent Preparation Log.

3.4.9.1 The lot number is TMX-MMDDYYYY

3.4.9.2 Solution expires one year from date of preparation.

3.4.9.3 Solution is kept refrigerated.

3.5 Calibration Standard Preparation

3.5.1 Prepare five alcohol calibration standards to the following concentrations:

3.5.1.1 Blank	diH_2O only	
3.5.1.2 STD A	0.005%	(1:4 dilution of STD B)
3.5.1.3 STD B	0.02%	(0.018 – 0.022 g/100 ml)
3.5.1.4 STD C	0.08 %	(0.072 – 0.088 g/100 ml)
3.5.1.5 STD D	0.20 %	(0.180 – 0.220 g/100 ml)
3.5.1.6 STD E	0.40 %	(0.360 – 0.440 g/100 ml)

3.5.2 Ethanol in diH_2O solutions are prepared gravimetrically in 100 ml volumetric flasks having the final concentrations as listed above (% wt./vol.).

3.5.2.1 Concentrations are rounded to three decimal places using 7 as a rounding value (i.e. 7 or above rounds up, 6 or below rounds down).

3.5.2.2 Standard C should be made in larger volumes as that solution is used as a CCS and will be used more frequently.

3.5.3 After preparation, the solutions are transferred to labeled screw-top vials.

3.5.4 The blank should be prepared using the same source diH_2O as the standards.

3.5.5 Document the standards in the Reagent Preparation Log.

3.5.5.1 The lot number is ES-MMDDYYYY.

3.5.5.2 Solutions expire three months from the date of preparation.

3.5.5.3 Solutions should be kept refrigerated.

3.5.5.4 The acceptance range for each prepared solution is +/- 10% from the ethanol concentration for all standards except STD A where the range is +/- 20%.

3.6 Purchased Calibrator and Control Verification

3.6.1 Aqueous Ethanol Control

3.6.1.1 NIST traceable aqueous ethanol standard is used for a within-run control. An aqueous ethanol standard with a concentration of 0.080 g/100mL is purchased from an ISO 17025 certified supplier. The certificates of analysis for all aqueous ethanol controls are kept on file with the Alcohol Program.

3.6.1.2 Prior to using a new lot of aqueous ethanol control, one vial from the lot should be run as a sample (in duplicate) to verify the lot falls within the manufacturer's specifications. A new shipment of the same lot does not require additional testing.

3.6.1.3 After a new lot of aqueous ethanol control is verified, the data package from the verification run will be technical reviewed and kept on file with the Alcohol Program.

3.6.1.3.1 Refer to ALC_P103_Alcohol Analysis Data Review and Reporting for review parameters.

3.6.2 Whole Blood Ethanol Control

3.6.2.1 A whole blood ethanol control is used for a within-run control. A whole blood control with an ethanol concentration of 0.080 g/100mL is purchased from a reputable supplier. The manufacturer control sheets for all whole blood ethanol controls are kept on file with the Alcohol Program.

3.6.2.2 Prior to using a new lot of whole blood ethanol controls or a new shipment of a previously received lot, ten (10) replicates of the control will be analyzed and the acceptable parameters determined.

3.6.2.2.1 The acceptance range will be +/- 10% from the calculated average.

3.6.2.3 After the parameters for a new lot of whole blood ethanol control have been determined, the data package from the analyses will be technical reviewed and kept on file with the Alcohol Program.

3.6.2.3.1 Refer to ALC_P103_Alcohol Analysis Data Review and Reporting for review parameters.

3.7 Sample Retrieval

3.7.1 Retrieve any samples having requested alcohol analysis from the Evidence intake refrigerator (Room 155A) and bring them to Room 266.

3.8 Opening Evidentiary Blood Kits and test tube labeling

3.8.1 Sample kits must be opened and the corresponding test tubes labeled one at a time at the lab bench.

3.8.2 Compare the information and laboratory identification number on the shipping box with the information in FA.

3.8.3 Take note of any identifying information written on any seals, on the test tubes or on the kit. If there is a discrepancy between that information and what is listed in FA, make a note in the comments section of FA. If there is a question regarding the identification of a sample, contact the Supervisor of the Alcohol Program.

3.8.4 Note the expiration date listed on the kit. If an expired kit has been used, make a note in the comments section of FA. It is permissible to use an expired kit; the expiration date refers to the vacuum of the blood tubes. If the tubes filled, regardless of the expiration date, the sample is deemed acceptable.

3.8.5 Remove the styrofoam holder from the cardboard box.

3.8.6 Open the styrofoam holder. Note in FA the number of tubes in the kit and whether or not each tube was sealed.

3.8.6.1 Any unsealed tubes will be sealed with evidence tape, dated and initialed. Make a note in the comments section of FA.

3.8.7 Label each test tube with the corresponding identification number (Ex: VFL# A1-1, A1-2, A1-3).

3.8.8 Repack the packaging material.

3.8.9 Repeat this process for all kits requiring alcohol analysis.

3.9 Headspace Standard Preparation

3.9.1 Remove the timing mix, standards, and whole blood ethanol control from the refrigerator in Room 266 and allow them to come to room temperature prior to preparation (approximately 30 minutes).

3.9.2 Check the “opened” date on the current vial of whole blood ethanol control (Level 1 0.08 EtOH from Cliniqa or equivalent). If the vial has been open for more than one month, dispose of it in the biohazard waste and retrieve a new vial. Allow the control to come to room temperature prior to sampling.

3.9.2.1 Ensure that the whole blood ethanol control has been verified prior to use.

3.9.3 Label one 20mL round bottom headspace auto sampler vial for each of the following:

3.9.3.1 Aqueous blank.

3.9.3.2 Timing mix.

3.9.3.3 Each of the five standards (A-E).

3.9.3.4 Whole blood ethanol control in duplicate.

3.9.3.5 A sufficient number of Calibration Check samples.

3.9.3.5.1 A calibration check sample using STD C is analyzed in duplicate after every 10^{th} vial and at the end of every run.

3.9.4 Transfer 1mL of internal standard to each headspace vial.

3.9.5 Add 500uL of each solution to its corresponding headspace vial.

3.9.5.1 When transferring the whole blood sample, use a wide bore, aerosol barrier pipette tip and work in the hood.

3.9.6 Using a cap crimping tool, seal the vials with a septum cap.

3.9.7 Analyze the standards as described in ALC_P102_Alcohol Analysis by Headspace GC-FID.

3.10 Headspace Blood Sample Preparation

3.10.1 Remove samples from the refrigerator in Room 266 and allow them to come to room temperature prior to preparation (approximately 30 minutes).

3.10.2 Evaluate all blood tubes submitted for quantity and condition of the sample. Each sample should contain at least 2mL of blood and be fluid enough to aliquot.

3.10.3 Select one test tube from the set for analysis and place it in a test tube rack.

3.10.4 Place all samples in the hood where aliquotting will take place.

3.10.5 Label two 20ml round bottom headspace auto sampler vials per sample with the VFL item #. All samples must be analyzed in duplicate.

3.10.6 Transfer 1mL of internal standard to each headspace vial.

3.10.7 Prior to aliquotting, vortex each blood sample for a minimum of 10 seconds to homogenize it.

3.10.8 Withdraw a 500uL aliquot of sample using a wide bore, aerosol barrier pipette tip and transfer the sample to the appropriate headspace vial. Discard the pipette tip into an autoclave bag.

3.10.9 Repeat for the duplicate sample.

3.10.10 Using a cap crimping tool, seal the vials with a septum cap.

3.10.11 Repeat this procedure for all samples in a batch.

3.10.12 Prior to analysis, samples can be held at room temperature for a maximum of 48 hours after preparation.

3.10.13 Analyze samples as described in ALC_P102_Alcohol Analysis by Headspace GC-FID.

3.11 Sample Storage and Retention

3.11.1 Subsequent to analysis, all samples are to be returned to the secure refrigerator for storage making sure to re-seal any tube that was opened for analysis.

3.11.2 Evidentiary blood tubes are kept for at least 90 days subsequent to analysis. They may be disposed of after that time.

3.12 Sample Analysis and Reporting

3.12.1 Refer to ALC_P102_Alcohol Analysis by Headspace GC-FID for instrument calibration and analysis procedures.

3.12.2 Refer to ALC_P103_Alcohol Analysis Data Review and Reporting for data compilation procedures.

4.0 Emergency or High Priority Situations

4.1 The Commissioner of Public Safety, Laboratory Director or Alcohol Program Supervisor can designate samples as high priority.

4.2 High priority samples are analyzed as soon as possible after successful calibration.

4.3 Priority sample results are reviewed and released as soon as they are available, once they pass the quality assurance criteria.

4.4 Any sample that is greater in concentration than the highest standard must be diluted and reanalyzed. The dilution recipe must be noted in FA worksheets.

4.5 Samples that arrive containing less than 2mL of blood may be analyzed with approval from the Alcohol Program Supervisor.

4.5.1 Low volume samples may be run as a half-prep using half the required sample and internal standard volumes. If alternate preparation is used, this must be documented in FA worksheets.

4.5.2 Effort should be made to preserve at least 500uL of sample for independent testing.

5.0 Quality Criteria and Corrective Action

5.1 Analysts will assure that an adequate amount of sample processing supplies are on hand at all times. Orders should be placed when supplies are low to ensure that new stock arrives before supplies are completely empty.

5.2 All analysts performing this procedure must analyze at least one set of proficiency samples per year. New analysts must complete an initial demonstration of capability.

6.0 Backup Procedures

6.1 If the secure storage refrigerator in room 155A is not functioning, the refrigerator in room 266 may be used to store samples, or vice versa.

6.2 If the Vermont Forensic Laboratory lacks analytical ability for greater than 10 business days, samples will be sent to a qualified reference lab for analysis. All submitters will be notified prior to submission of samples for outside analysis.

7.0 References

7.1 ALC_P102_Alcohol Analysis by Headspace GC-FID

7.2 ALC_P103_Alcohol Analysis Data Review and Reporting

DOCUMENT HISTORY			
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